Phytochemical screening, GCMS, UV-vis and FTIR analysis of leaf methanolic extract of *Sapindus mukorossi* L

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Abstract: - *Sapindus mukarossi* L is a medicinal plant comprising health-promoting secondary metabolites. The present study was aimed to analyses the phytochemicals through preliminary phytochemical screening and also to profile bioactive compounds in the methanolic extract of *S. mukorossi* leaves using GCMS, UV-vis, FTIR. Thin-layer chromatography (TLC) was carried out for the comparative metabolite profiling of the extracts using Chloroform: Methanol (90:10v/v) as a solvent system. The preliminary phytochemical screening tests revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, carbohydrates. The GCMS analysis of methanolic leaf extract was performed to identify the various bioactive compounds based on separation and concentration. FTIR analysis was done to identify the functional groups present in the plant extract. The UV-vis profile showed the presence of peaks which reveals the presence of secondary metabolites. From this study, it is concluded that the plant species contain effective phytochemical compounds, needs further research on toxicological aspects to develop a safe drug.

Key Words: — ***Sapindus mukorossi, Methanol, TLC, FTIR, GCMS, UV-vis, Phytochemicals.***

I. INTRODUCTION

Nowadays traditional system of medicine is widely used for the treatment of many diseases. Medicinal plants are said to be much safer and proved in the treatment of various ailments. They show importance in the primary health care of individuals and communities in many developing countries. There has been an increase in demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as an alternative to allopathic medicines (Ashis et al., 2003). Plants produce bioactive compounds in a diverse range making them a rich source of different types of medicines (Jeeva and Johnson, 2012; Florence et al., 2014). Traditionally, plant extracts were known to be effective against microorganisms as a result; plants form the basis of modern medicine.

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Plants produce phytochemicals to protect themselves; but recent studies indicate that many phytochemicals can also protect humans against infectious diseases (Florence et al., 2014; Domettila et al., 2013).

Sapindus mukorossi L, is a large deciduous tree which belongs to the family Sapindaceae and grows upto to 12 meters height. It is well known for its folk medicinal values (Sharma et al, 2011). The fruits are of considerable importance for their medicinal value for treating a number of diseases like excessive salivation, pimples, epilepsy, chlorosis, migraines, eczema and psoriasis (Kirtikar et al., 1991).

Within a decade, there were several dramatic advances in analytical techniques including HPLC, UV, FTIR, GCMS and NMR that were powerful tools for separation, identification, and structure determination of phytochemicals (Robert et al., 1995). The present study aims to determine the bioactive compounds present in the S. mukorossi plant extract with the aid of TLC, GCMS, UV-vis, and FTIR techniques, which may provide an insight into its use of traditional medicine.

II. MATERIALS AND METHODS

2.1. Collection of plant material

The plant *S. mukorossi* was collected from Targal, area of Hulekal, Uttar Kannada district of Karnataka during the month of January 2022. Collected leaves were surface sterilized for 3 minutes using 0.01% mercuric chloride followed by 70% ethyl alcohol for 1 minute. Surface sterilized leaves were rinsed using sterile double distilled water and dried using aseptic blotter paper.

2.2 Preparation of extracts

The shade dried leaves of *S. mukorossi* were coarse powdered. About 50 grams of dried coarse powder was subjected to methanol extraction as a solvent at 40˚C by continuous hot extraction method.

2.3 Preliminary phytochemical screening

Various chemical tests were carried out on the Methanolic extract using standard procedures to identify the preliminary screening as per the Harborne,1973 and Sofowara,1993.

2.4 Alkaloids (Mayer's test)

To 2ml of leaf extract, 1ml of dilute HCl is added and a few drops of Mayer's reagent were added along the slide of the test tube. The formation of yellow colour indicates the presence of alkaloids.

2.5 Flavonoids (NaOH test)

The leaf extract was dissolved in 2ml of 2% NaOH and further observed for the formation of intense yellow colour which becomes colorless on addition of few drops of dil. HCl indicates the presence of Flavonoids. (Arusle *et al*., 2017)

2.6 Phenol (Lead acetate test)

The leaf extract was dissolved in 5ml of distilled water and 3ml of 10% lead acetate solution. The formation of white precipitate indicates the presence of Phenolic compounds. (Raaman *et al*.,2006)

2.7 Lignin (Labat test)

To the leaf extract gallic acid is added. The appearance of olive-green colour indicates the presence of lignin. (Bhatt *et al*., 2012.Nanna *et al*., 2013).

2.8 Sterols (Salkowski's test)

Few drops of chloroform are added to the methanolic extract along with few drops of sulfuric acid. The appearance of greenish yellow fluorescence indicates the presence of Sterols.

2.9 Saponin (Foam test)

1ml of methanolic extract was added to distilled water and shaken vigorously until the formation of honeycomb like foam for 10 to 15minutes indicates the presence of Saponin.

2.10 Terpenoids (Salkowski's test)

Few drops of chloroform are added to the methanolic extract along with few drops of sulfuric acid. The appearance of radish brown colour indicates the presence of Terpenoids. (Das Kunatal *et al*., 2015).

2.11 Tannins (Ferric chloride test)

To 2ml of leaf extract, the ferric chloride solution was added. The formation of black precipitate indicates the presences of tannins.

2.12 Glycosides (Keller Killani test)

To 1ml of filtrate, 1.5ml of glacial acetic acid was added to this 1 drop of 5% ferric chloride and concentrated H_2SO_4 along the sides of test tube. Appearance of reddish brown indicates the presence of Glycosides.

2.13 Reducing Sugar (Benedict's test)

1ml of methanolic extract and 2ml Benedict's reagent solution was heated in boiling water bath for 3 to 5 minutes. The presence of reducing sugar is indicated by the formation of green colour. (Raaman *et al*., 2006, Singh *et al*., 2017).

2.14 Carbohydrate (Molisch's test)

To 2ml of methanolic extract α-napthol is added. Further concentrated H_2SO_4 along the sides of the test tube. The violet ring formation at the junction indicates the presence of Carbohydrates. (Raaman *et al*., 2006, Singh *et al*., 2017)

2.15 Proteins (Ninhydrin test)

To 2ml of leaf extract, few drops of 2% ninhydrin solution is added. The violet colour formation indicates the presence of Proteins. (Raaman *et al*., 2006, Silva *et al*., 2017)

2.16 Thin Layer Chromatography (TLC).

2.16.1 Qualitative analysis by thin layer chromatography

TLC studies were carried out to select the solvent system capable of showing better resolution and different solvent systems of different polarities were prepared. Analytical TLC of the methanolic extract was carried out using a silica TLC plate (60 F_{254}, 20×20cm, 0.25mm thick, Merck). The organic extract was used and applied onto silica gel TLC plates.

Chloroform: Methanol (90:10) was used as mobile phase. The plates were observed using iodine crystals as the detection system. Alternatively, the TLC plates were also observed under UV light. Plant sample (10 µl) were loaded on TLC plate as line spots.

Capillary tubes were used while applying plant extracts on pre-coated TLC plates using suitable mobile phase. After color development, the plates were air dried and also observed using UV light. Values were determined using retention factor and calculated (Takeuchi *et al.,* 1991).

$$R_f = \frac{\text{Distance travelled by solute front}}{\text{Distance travelled by the solvent front}}$$

Where; Rf- Retention factor

2.17 UV-vis and FTIR Spectroscopic analysis.

The extracts were examined under UV visible light for proximate analysis. For UV-vis and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No.1 filter paper by using a high-pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-800 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 1000-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

2.18 GCMS Analysis.

GCMS analysis of methanolic extract were performed using a Perkin - Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GCMS) equipped with an Elite - 1, fused silica capillary column (30 mm x 0.25mm 10 x 1 µMdF, composed of 100% di methyl poly siloxane). For GCMS detection an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2µ1 was employed (split ratio of 10:1); injector temperature 2500ºC; ion-source temperature 2800ºC. The oven temperature programmed from 1100ºC (isothermal for 2 min) with an increase of 100ºC/min to 2000ºC, then 50ºC/min to 2800ºC, ending with a 9 min isothermal at 2800ºC, mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da, total GC running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo mass.

Interpretation on mass spectrum GC-MS was done using the database of National Institute of Standard and Technology (NIST) library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name molecular weight and structure of the components of the test materials were ascertained.

III. RESULTS

3.1 Collection of plant material

The plant *S. mukarossi* was collected from from Targal, area of Hulekal, Uttar kannada district of Karnataka during the month of January 2022. (Fig 1). The plant was identified by the taxonomist Dr. Kotresha, Associate Professor, Department of Botany, Karnatak University, Dharwad. The area being at a latitude of 14.619500 N and longitude at 74.835403 E. The leaves were dried in shade condition. Then the shade dried leaves were made into coarse powder and were used for different investigations.

Fig.1. *S. mukorossi* plant

3.2 Qualitative detection of secondary metabolites

Leaf methanolic extract of *S. mukorossi* plant, were found to be able to produce all the functional metabolites (table 1). All the isolates showed more or less efficient (as observed from the intensity of color) for the production of alkaloids, flavonoids, saponins, sterols, carbohydrates, terpenoids and reducing sugars (Fig 2).

Table.1. Qualitative detection of secondary metabolites from *S. mukorossi* (leaf extract)

Phytochemical Test	Methanolic leaf extract
Alkaloids	+
Flavonoids	+
Phenol	+
Lignin	+
Sterols	+
Saponins	+
Terpenoids	+
Tannin	-
Glycosides	+
Reducing Sugar	+
Carbohydrates	+
Protein	-

+ ⟶ Presence; - ⟶ Absence.

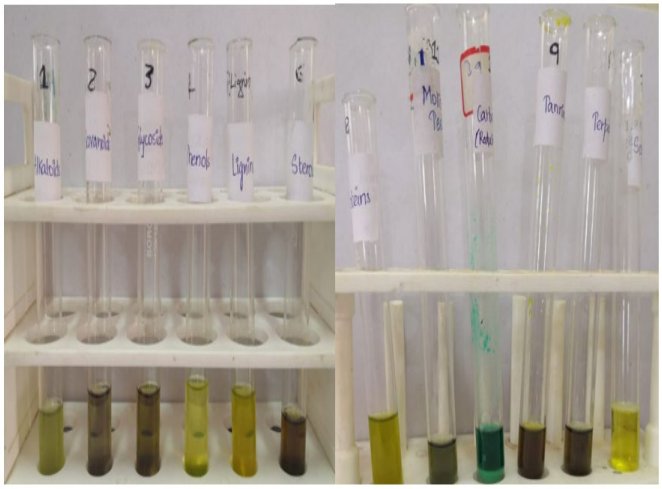


Fig.2. Isolation and identification of secondary metabolites from *S. mukorossi* (leaf extract)

3.3 Qualitative analysis by thin layer chromatography.

The Rf values of the purified plant extract obtained from *S. mukorossi* were determined. Rf value was found to be 0.92 TLC plate using solvent Choloform: methanol (90:10 v/v) which has been used as mobile phase (Fig 3).

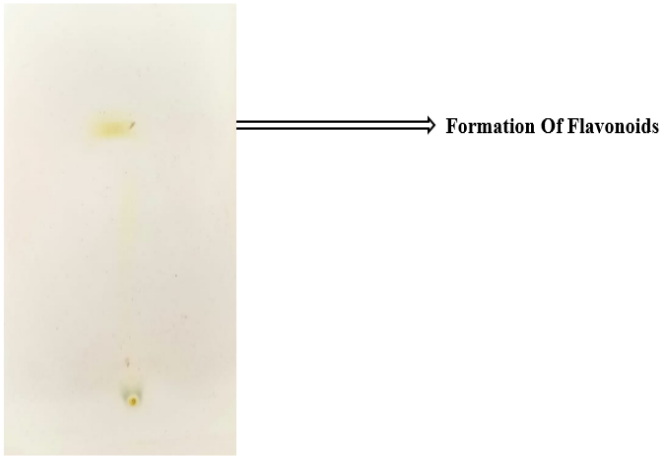


Fig.3. TLC plate showing formation of flavonoids (Yellow color)

3.4 Gas Chromatography Mass Spectroscopy of purified methanol leaf extract of plant

The methanol extract of leaf was partially purified by TLC analysis. The partially purified crude extract was subjected to GC-MS analysis which shows retention time, area %, molecular formula and molecular weight of the several compounds were identified and tabulated (Fig 4). The gas chromatography results of crude extract reveal the major active compounds which are present in the plants (table 2).

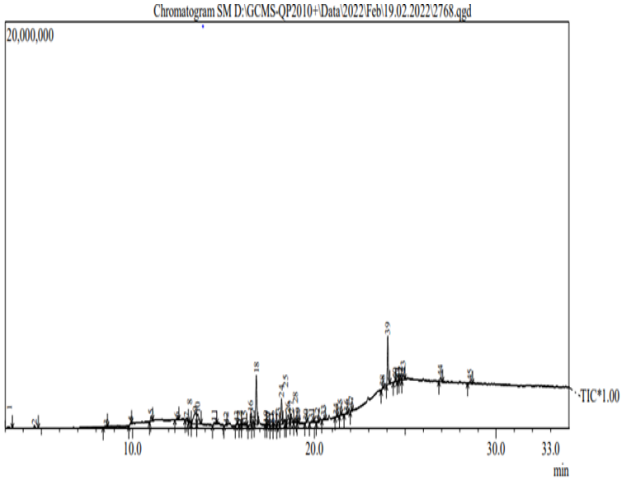
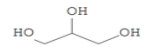
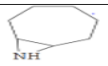
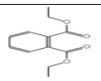
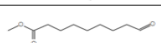
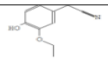
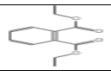
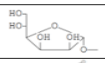
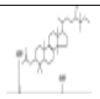
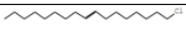
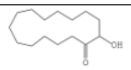
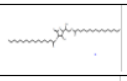
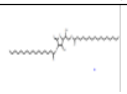
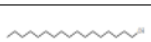
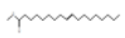
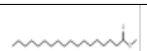
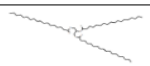
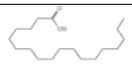
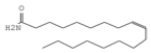
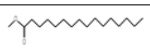
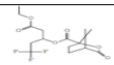
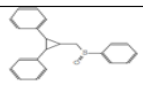
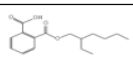
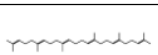
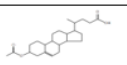
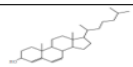
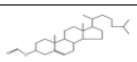
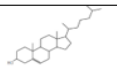
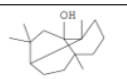


Fig.4. GCMS Chromatogram of *S. mukorossi*

Table 2: Different bioactive compounds showing in leaf methanolic extract of *S. mukorossi*

Component No	RT	Ligands	Formula	M W	Structure	CAS#
1	3.152	Cyclopropane acetic acid, 2-hexyl-	$C_{11}H_{20}O_2$	184		35936-15-3
2	4.600	Glycerin	$C_3H_8O_3$	92		56-81-5
3	8.558	8-Azabicyclo [5.1.0] octane	$C_7H_{13}N$	111		286-44-2
4	9.910	Diethyl Phthalate	$C_{12}H_{14}O_4$	222		84-66-2
5	11.011	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186		1931-63-1
6	12.435	3-Ethoxy-4-hydroxyphenylacetonitrile	$C_{10}H_{11}NO_2$	177		7779-17-1
7	12.979	Diethyl Phthalate	$C_{12}H_{14}O_4$	222		84-66-2
8	13.139	Decanal	$C_{10}H_{20}O$	156		112-31-2
9	13.451	alpha. -d-Mannofuranoside, methyl	$C_7H_{14}O_6$	194		4097-91-0
10	13.596	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194		0-00-0
11	14.493	Tetradecanoic acid	$C_{14}H_{28}O_2$	228		:544-63-8
12	15.149	Acetic acid, 17-(4-hydroxy-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4	$C_{33}H_{56}O_4$	516		0-00-0
13	15.723	Pentadecanoic acid	$C_{15}H_{30}O_2$	242		002-84-2
14	15.948	8-Heptadecene, 1-chloro-	$C_{17}H_{33}Cl$	272		56554-80-4
15	16.145	Diethylene glycol monododecyl ether	$C_{16}H_{34}O_3$	274		:3055-93-4
16	16.497	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270		112-39-0
17	16.628	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240		4727-18-8
18	16.810	0 l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652		28474-90-0
19	17.336	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652		28474-90-0
20	17.431	4-[1,2-Dimethyl-6-(2-trimethylsilylethoxymethoxy)cyclohexyl]but-3-en-2-one	$C_{18}H_{34}O_3Si$	326		119018-20-1
21	17.655	1-Heptadecanol	$C_{17}H_{36}O$	256		1454-85-9
22	17.796	Hexanoic acid, octadecyl ester	$C_{24}H_{48}O_2$	368		41927-67-7

23	18.018	9-Tricosene, (Z)-	$C_{23}H_{46}$	322		27519-02-4
24	18.197	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296		1937-62-8
25	18.409	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298		112-61-8
26	18.552	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	$C_{57}H_{104}O_6$	884		537-39-3
27	18.721	Octadecanoic acid	$C_{18}H_{36}O_2$	284		57-11-4
28	18.950	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281		301-02-0
29	19.094	Phenol, 2,4'-isopropylidenedi-	$C_{15}H_{16}O_2$	228		837-08-1
30	19.536	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{17}H_{34}O_2$	270		112-39-0
31	19.846	1-Nonadecene	$C_{19}H_{38}$	266		18435-45-5
32	20.175	Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	396		55373-89-2
33	20.509	7,7-Dimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carboxylic acid, 1-ethoxycarbonylmeth	$C_{16}H_{21}F_3O_6$	366		0-00-0
34	21.199	cis-1-Chloro-9-octadecene	$C_{18}H_{35}Cl$	286		16507-61-2

35	21.433	2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	$C_{22}H_{20}OS$	332		131758-71-9
36	21.781	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354		929-77-1
37	22.031	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278		4376-20-9
38	23.744	Hexacontane	$C_{60}H_{122}$	842		7667-80-3
39	24.039	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)	$C_{30}H_{50}$	410		111-02-4
40	24.431	Tetracontane	$C_{40}H_{82}$	562		4181-95-7
41	24.610	3.beta.-Acetoxy-5-bisnorcholenic acid	$C_{24}H_{36}O_4$	388		0-00-0
42	24.728	Cholesta-4,6-dien-3-ol, (3.beta.)-	$C_{27}H_{44}O$	384		14214-69-8
43	24.884	Cholesteryl formate	$C_{28}H_{46}O_2$	414		4351-55-7
44	26.928	Cholest-5-en-3-ol (3.beta.)-	$C_{27}H_{46}O$	386		57-88-5
45	28.552	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	$C_{15}H_{26}O$	222		117591-80-7

3.5 FTIR Spectroscopic analysis

Infrared spectroscopy involves the absorption of electromagnetic radiation in the infrared region of the spectrum which results in changes in the vibrational energy of molecules. Since, all the molecules have vibrations in the form of stretching, bending etc., the absorbed energy will be utilized in changing the energy levels associated with them.

3.6 Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peak's values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peak's ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, alkenes, alcohol, aromatic, aliphatic amines and amine compound in all the four seasons. The leaf methanolic extracts are taken for the FTIR techniques (Fig. 5 and Table 3).

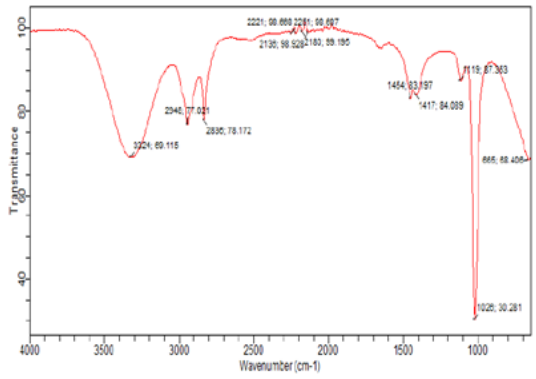


Fig.5. FTIR Spectra of Leaf Methanolic extract of *S. mukarossi*

Table.3. FTIR Interpretation of compounds of Leaf Methanolic extract of *S. mukorossi*

SL.NO.	Frequency (Cm^{-1})	Bond	Functional Groups
1.	3324	O–H Stretch, H–Bonded	Alcohols, Phenols
2.	2836-2948	C–H Stretch	Alkanes
3.	2180	–C=C– Stretch	Alkenes
4.	1119-1417	C–C Stretch (In–Ring)	Aromatics
5.	1026	C–N Stretch	Aliphatic Amines
6.	665	N–H Wag	1°, 2° Amine

3.7 UV-Visible Spectrometry

The UV-vis profile of plant extract was taken at the 200 to 800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings (Fig. 6). The precise position and relative intensities of these maxima give valuable information on the nature of the secondary metabolites. Occurrence of peaks of plant extracts reveals the presence of secondary metabolites in the *S. mukorossi*.

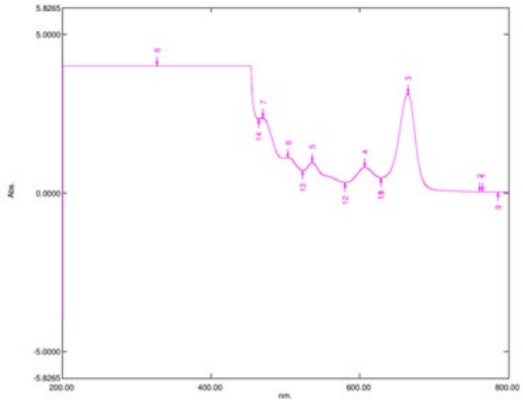


Fig.6. UV-vis spectra of Leaf Methanolic extract of *S. mukorossi*

IV. Conclusion

The study concluded that the methanolic extract of *S. mukorossi* has potential bioactive compounds like alkaloids, flavonoids, tannins, etc. TLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The phytochemical screening shows that the presence of secondary metabolites. FTIR analysis confirmed the presence of phenol, alkanes, alkenes, alcohol, aromatic, aliphatic amines and amine compound. Gas chromatography Mass spectroscopy (GC-MS) is a valuable tool for reliable identification of phytocompounds (Sampathkumar *et al.,* 2011, Johnson *et al.,* 2011, Balamurugan *et al.,* 2011). In the present study, 45 compounds have been identified from the methanolic extract of the leaf of *S.mukorossi* by GC-MS analysis. However further studies will need to be undertaken to isolate and screening of bioactive compounds from methanol extract of aerial parts and find out its biological activity.

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